Evaluation of diagnostic value of a recombinant protein mimicking envelop glycoprotein gp105 of human immunodeficiency virus type 2 (HIV2) in the detection of anti-HIV2 antibodies in linear immunoblot (LIA) format

N.V. Chepurchenko, N.V. Saveleva, N.V. Zabegalov, A.P. Obriadiia
Research Department, RPC Diagnostic Systems, Nizhny Novgorod, Russia. http://www.npods.ru, natacv@npods.ru +7(831) 434-86-83

#P0742

**Background**

Detection of antibodies against envelope glycoprotein (env) gp105 is an essential requirement by WHO for diagnosis of human immunodeficiency virus type 2 (HIV2). Immunoblotting (Western blot or Line Immuno Assay (LIA)) is recommended as a confirmatory test. Applying of HIV2 gp105 onto 0.45nm membrane in form of 22 amino acid (aa) long synthetic peptide was complicated due to its small size. Immobilization of a biotinylated form of the peptide through streptavidin molecule has overcome the size problem, but decreased the specificity of the LIA test. The attempt to present the peptide sequence in a form of a tagged recombinant protein was taken.

**Materials/Methods**

Region 306-327 aa of HIV2 envelop protein has been amplified by PCR and cloned as a recombinant protein in fusion with GST+6His tag. A second recombinant protein with the doubled target 22aa-sequence was also constructed. The obtained proteins have been immobilized on a nitrocellulose membrane and tested against 52 HIV-2 positive (Biocentric, France) and 132 HIV-negative ( donor plasmas, Russia, Volgo-Vyatka region) samples in LIA format in comparison with the synthetic form of the peptide. LIA format: incubation at room temperature under shaking 1) with samples for 2 hours, 2) with mouse alkaline phosphatase-labeled anti-human IgG (conjugate) 1 hour, 3) with BSIP/NBT substrate 10 minutes.

**Results**

Sensitivity of the LIA test with gp 105 in the form of biotinylated synthetic peptide was 79.5%, specificity was 87.3%; non-specific reactions of serum components with streptavidin occurred. Recombinant protein with one repeat of the 22aa sequence in composition appeared to be inefficient in anti-HIV2 detection. The recombinant carrying the dimer version of the 22aa-peptide sequence has increased the specificity of LIA test up to 98.4%, and the sensitivity remained the same.

**Conclusion**

Redouble of the target 22aa sequence of HIV2gp105 in the composition of a recombinant protein with GST+6His tag has allowed direct sorption on the nitrocellulose membrane, and has significantly increased specificity of anti-HIV Ab detection in LIA format due to correct presentation of the immunodominant epitope.

![Diagram of HIV-2 envelope protein](https://www.ab-ds.de/raw-materials/npods.ru)

**Table 1**

<table>
<thead>
<tr>
<th>gp105</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptide</td>
<td>79.5%</td>
<td>87.3%</td>
</tr>
<tr>
<td>Recombinant protein (monomer)</td>
<td>63.6%</td>
<td>92.8%</td>
</tr>
<tr>
<td>Recombinant protein (dimer)</td>
<td>79.5%</td>
<td>98.4%</td>
</tr>
</tbody>
</table>

**International distribution by AB “Diagnostic Systems”:**
https://www.ab-ds.de raw-materials@npods.ru +7(831) 467-82-15 (ext.7685)